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Claims

1. A method of differentiating HCV genotype 1 (HCV-1) from HCV genotypes 2 and 3 (HCV-2 and HCV-3) in a sample, comprising:

subjecting the sample to an amplification reaction using at least one primer which anneals specifically to the 5' noncoding region (5' NCR) of the HCV-1 genome; and

detecting the product of the amplification reaction.

2. A method as claimed in claim 1, wherein the amplification reaction is the polymerase chain reaction (PCR) or reverse transcriptase polymerase chain reaction (RT-PCR).

3. A method as claimed in claim 1 or claim 2, further comprising:

detecting whether any HCV genotype is present in the sample by subjecting the sample to an amplification reaction using primers which anneal to a region of the 5'NCR which is conserved between all HCV genotypes; and

detecting the product of this amplification reaction.

4. A method as claimed in claim 3, wherein the primers have the following sequences:

Forward: 5' CGT CTA GCC ATG GCG TTA G 3' *(UTR-L2)*

Reverse: 5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

5. A method as claimed in any preceding claim, further comprising subjecting the sample to a preliminary amplification reaction to isolate HCV material using primers universal for all HCV genotypes.

6. A method as claimed in claim 5, wherein the primers comprise the following sequences:

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Forward: 5' GGA ACT ACT GTC TTC ACG C 3' *(UTR-L1)*

Reverse: 5' ACG GTC TAC GAG ACC TC 3' *(UTR-R1)*

7. A method as claimed in any preceding claim, wherein the at least one primer which anneals specifically to the 5' noncoding region (5' NCR) of the HCV-1 genome comprises the sequence:

5' CCI CTC AAT GCC TGG AG . 3' *(Spec-1)*.

8. A method as claimed in claim 7, wherein the at least one primer which anneals specifically to the 5' noncoding region (5' NCR) of the HCV-1 genome is a forward primer and the reverse primer comprises the sequence:

5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

9. A method as claimed in any preceding claim, wherein detection of the product of the or each amplification reaction is by agarose gel electrophoresis.

10. A method as claimed in any one of claims 1 to 8, wherein detection of the product of the or each amplification reaction is by fluorescent analysis in which amplification of HCV-1 specific nucleic acid causes fluorescence of a probe.

11. A method as claimed in claim 10, wherein the probe comprises the sequence:

5' FCG CIA CCC AAC ICT ACT IGG CTA GT 3' *(L1)*

where F=6-FAM,3'-T+TAMRA.

12. A method as claimed in any one of claims 1 to 6, wherein detection of the product of the or each amplification reaction is by one or more molecular beacon primers.

13. A method as claimed in claim 12, wherein the molecular beacon primer comprises the sequence:

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5' FCA CCT TCA CCC TCA GAA GGM GCC GCT CAA TGC CTG GAG 3' (F=FAM; M=MeREDdU and U=Uracil) *(MBP-LR-1)*

14. A method as claimed in claim 13, wherein the molecular beacon primer is a forward primer and the reverse primer comprises the sequence:

5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

15. A method as claimed in claim 12, 13 or 14 when appended to claim 2, wherein the primers which anneal to a region of the 5'NCR which is conserved between all HCV genotypes comprise the following sequences:

Forward: 5' FCA CCT TCA CCC TCA GAA GGM GCG UCT AGC CAT GGC GTT AG 3' (F=FAM; M=MeREDdU and U=Uracil) *MBP-LR-ALL*

Reverse: 5' GCA GTA CCA CAA GGC CTT TCG C 3' (UTR-R2)

16. A kit for detecting HCV genotype 1 (HCV-1) in a sample, comprising: at least one primer which anneals specifically to the 5' noncoding region (5' NCR) of the HCV-1 genome.

17. A kit as claimed in claim 16, modified by the features of any one of claims 2 to 16.

18. A nucleotide molecule suitable for use in an amplification reaction comprising one of the following sequences:

5' CGT CTA GCC ATG GCG TTA G 3' *(UTR-L2)*

5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

5' GGA ACT ACT GTC TTC ACG C 3' *(UTR-L1)*

5' ACG GTC TAC GAG ACC TC 3' *(UTR-R1)*

5' CCI CTC AAT GCC TGG AG 3' *(Spec-1)*.

5' FCA CCT TCA CCC TCA GAA GGM GCC GCT CAA TGC CTG GAG3' (F=FAM; M=MeREDdU and U=Uracil) *(MBP-LR-1)*

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5' FCA CCT TCA CCC TCA GAA GGM GCG UCT AGC CAT GGC GTT AG 3' (F=FAM; M=MeREDdU and U=Uracil) *MBP-LR-ALL*

19. A pair of primers comprising a nucleotide molecule having the following sequence:

Forward: 5' CGT CTA GCC ATG GCG TTA G 3' *(UTR-L2)*

Reverse: 5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

Forward: 5' GGA ACT ACT GTC TTC ACG C 3' *(UTR-L1)*

Reverse: 5' ACG GTC TAC GAG ACC TC 3' *(UTR-R1)*

Forward: 5' CCI CTC AAT GCC TGG AG 3' *(Spec-1)*.

Reverse 5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

Forward: 5'FCA CCT TCA CCC TCA GAA GGM GCC GCT CAA TGC CTG GAG3' (F=FAM; M=MeREDdU and U=Uracil) *(MBP-LR-1)*

Reverse: 5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

Forward: 5' FCA CCT TCA CCC TCA GAA GGM GCG UCT AGC CAT GGC GTT AG 3' (F=FAM; M=MeREDdU and U=Uracil) *MBP-LR-ALL*

Reverse: 5' GCA GTA CCA CAA GGC CTT TCG C 3' *(UTR-R2)*

20. A nucleotide molecule suitable for use as a probe comprising the sequence:

5'FCG CIA CCC AAC ICT ACT IGG CTA GT3' *(L1)*

(where F=6-FAM,3'-T+TAMRA).

21. A method of differentiating HCV genotype 1 (HCV-1) from HCV genotypes 2 and 3 (HCV-2 and HCV-3) in a sample, comprising:

subjecting the sample to an amplification reaction using at least one primer which anneals to the genome of HCV, a polymerase having a 5'-3' exonuclease activity and an

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oligonucleotide probe, which probe anneals specifically to the 5' noncoding region (5' NCR) of the HCV-1 genome and which incorporates a modified nucleotide having a fluorescent characteristic which is modified by one or more neighbouring nucleotides; and

detecting a change in fluorescence as the oligonucleotide probe is degraded by the exonuclease activity of the polymerase as the polymerase extends the primer and modification of the fluorescent characteristic of the modified nucleotide is reduced.